# Fast Whole Genome Amplification from Single Cells or Purified gDNA Using the REPLI-g® Single Cell Kit

This protocol is for the amplification of genomic DNA from 1–1000 intact cells of vertebrates, including sorted cells and cells from tissue culture or bacteria (gram-positive and gram-negative) – or from >6 pg purified gDNA from any eukaryotes, including plants, fungi, bacteria or viruses. It uses a fast 3 h incubation time and results in up to 30 µg of amplified DNA per reaction.

**IMPORTANT**: Please read the handbooks supplied with the REPLI-g Single Cell Kit, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. The REPLI-g Single Cell Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

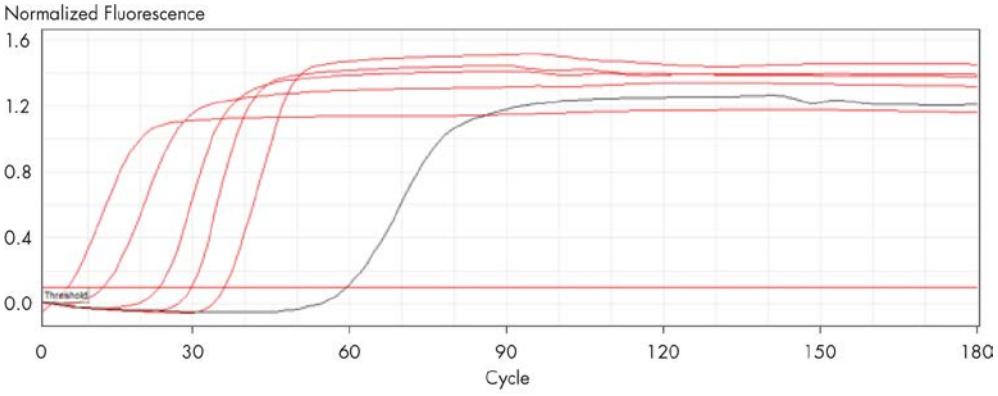


**Figure 1. Real-time amplification of 1 ng, 100 pg, 10 pg, 1 pg and 100 fg of human gDNA (red) and NTC ( black).** REPLI-g reactions were performed with additional EvaGreen® Dye in a Rotor-Gene® to track amplification in real-time. Reaction time (in minutes) is presented on the x-axis. Additional data based on end-point quantification indicates that yield increases until up to 3 h. The plateau visible after 1 h, as seen in the figure, is due to limiting reaction components.

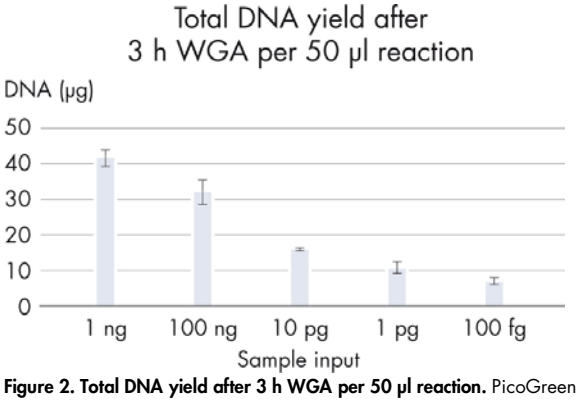


**Figure 2. Total DNA yield after 3 h WGA per 50 µl reaction.** PicoGreen quantification of REPLI-g amplified DNA was performed according to protocol in the *REPLI-g Single Cell Handbook*.

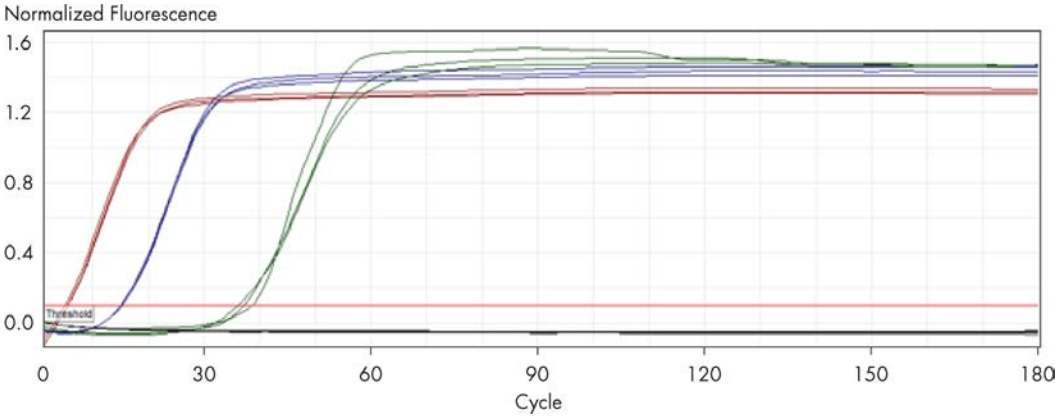


**Figure 3. Real-time amplification of 500 cells (red) – 50 cells (blue) – 5 cells (green) – 0 cells = NTC (black) human cells.**

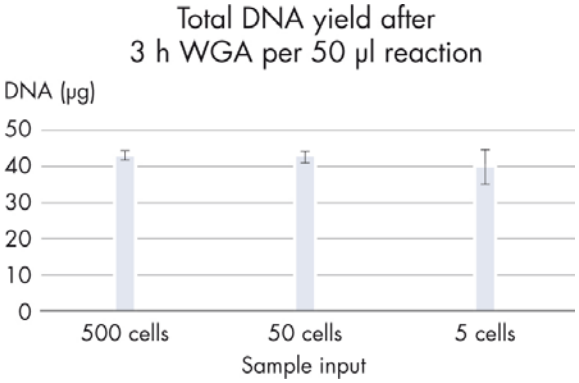


**Figure 4. Total DNA yield after 3 h WGA per 50 µl reaction.** PicoGreen quantification of REPLI-g amplified DNA was performed according to protocol in the *REPLI-g Single Cell Handbook*.

## Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- REPLI-g Single Cell Kit (QIAGEN, cat. no. 150343 for 24 preps; cat. no. 150345 for 96 preps)
- Microcentrifuge tubes
- Water bath or heating block
- Vortexer
- Microcentrifuge
- Pipets and pipet tips
- Ice

## Important points before starting

- This protocol is optimized for single cell material from all species, including: vertebrates, bacteria (gram-positive and gram-negative), flow-sorted cells, tissue culture cells, micromanipulated cells and laser-microdissected cells from frozen sections. The protocol cannot be used with cells fixed with formalin or other cross-linking agents (e.g., single cell samples obtained by laser microdissection from formalin-fixed, paraffin-embedded tissues).
- Samples of 1–1000 intact cells (e.g., eukaryotic or bacterial cells) are optimal for whole genome amplification reactions using the REPLI-g Single Cell Kit.
- If starting with purified DNA rather than intact cells, ensure that the DNA is suspended in TE buffer and is of high quality (having high molecular weight without the presence of inhibitors, such as solvents and detergents). If working with eukaryotic DNA, we recommend using 1–10 ng; for bacterial gDNA, we recommend using 6–100 pg.
- Avoid DNA contamination of reagents by using separate laboratory equipment (e.g., pipets, filter pipet tips, reaction vials, etc.). Set up the REPLI-g Single Cell reaction in a location free of DNA.
- REPLI-g sc DNA Polymerase should be thawed on ice (see step 8). All other components can be thawed at room temperature (15–25°C).
- Buffer D2 (denaturation buffer) should not be stored for longer than 3 months.
- DNA yields of up to 20 µg may be present in negative (no-template) controls, as DNA is generated during the REPLI-g Single Cell reaction by random extension of primer dimers – generating high-molecular-weight products. This DNA will not affect the quality of the actual samples and will not give a positive result in downstream assays.

## Things to do before starting

- Prepare Buffer DLB by adding 500 µl $H_2O$ sc to the tube provided. Mix thoroughly and centrifuge briefly to dissolve.

  **Note**: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile.
- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- Set a programmable thermal cycler, or a heating block, to 30°C.
- If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.

## Procedure

1. Thaw $H_2O$ sc, DTT and REPLI-g sc reaction buffer at room temperature; vortex, then centrifuge briefly. The REPLI-g sc Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
2. Prepare sufficient Buffer D2 (denaturation buffer) for the total number of whole genome amplification reactions (Table 1).

   **Note**: The total volume of Buffer D2 given in Table 1 is sufficient for 12 reactions. If performing fewer reactions, store residual Buffer D2 at –20°C. Buffer D2 should not be stored longer than 3 months.

**Table 1. Preparation of Buffer D2 for 12 reactions**

| Component | Volume for 12 reactions |
|---|---|
| DTT, 1 M | 3 µl |
| Buffer DLB (reconstituted)* | 33 µl |
| **Total volume** | **36 µl** |

* Reconstitution of Buffer DLB is described in "Things to do before starting," page 3.

3. Prepare sufficient 1:10 dilution DTT in $H_2O$ sc for the total number of reactions (Table 2).

**Table 2. Preparation of DTT 1:10 dilution for 12 reactions**

| Component | Volume for 12 reactions* |
|---|---|
| DTT, 1 M | 3 µl |
| $H_20$ sc | 27 µl |
| **Total volume** | **30 µl** |

* Add 10%.

4. Place 4 µl cell material (supplied with PBS) or gDNA into each well of a 96-well plate or microcentrifuge tube. If using less than 4 µl of starting material, add PBS sc to bring the volume up to 4 µl.

   **Note**: During pipetting, avoid contact of pipet tips and cell material.
5. Add 3 µl Buffer D2. Mix carefully by gently flicking the tube and centrifuge briefly.

   **Note**: Ensure that the cell material does not stick to the tube wall above the buffer line. During pipetting, avoid any contact of pipet tips with cell material.
6. Incubate cell preparations for 10 min at 65°C and gDNA preparations for 3 min at room temperature; then cool down to 4°C.

**Note**: If a thermal cycler is used, the temperature of the heating lid should be set at 70°C to avoid evaporation. Alternatively, incubation can be performed in a heating block.

7. Add 3 µl Stop Solution. Mix carefully by flicking the tube and centrifuge briefly. Store on ice.
8. Thaw REPLI-g sc DNA Polymerase on ice. Tip the tube gently to mix, then centrifuge briefly.
9. Prepare a master mix (Table 3). Mix and centrifuge briefly.

   **IMPORTANT**: Add the master mix components in the order listed in Table 3. After the addition of water, REPLI-g sc Reaction Buffer and DTT, briefly vortex and centrifuge the mixture before adding REPLI-g sc DNA Polymerase. After adding REPLI-g sc DNA Polymerase, flick carefully and centrifuge briefly. The master mix should be kept on ice and used immediately upon the addition of REPLI-g sc DNA Polymerase.

   **Note**: Scale up accordingly if performing several reactions at once by preparing a master mix sufficient for the total number of reactions.

**Table 3. Preparation of master mix***

| Component | Volume for 12 reactions* |
|---|---|
| $H_20$ sc | 6.5 µl |
| REPLI-g sc Reaction Buffer | 29 µl |
| DTT (1:10) | 2.5 µl |
| REPLI-g sc DNA Polymerase | 2 µl |
| **Total volume** | **40 µl** |

* To prepare a master mix for multiple reactions, scale up according to the number of reactions and add 10%.

**Note**: Add 2 µl of EvaGreen (25x) to perform real-time amplification. If EvaGreen is added, then add only 4.5 µl of water to Reaction Buffer and DTT; briefly vortex and centrifuge the mixture before adding REPLI-g sc Polymerase.

10. For each amplification reaction, add 40 µl master mix to 10 µl denatured DNA (from step 7). Mix by flicking the tube, and centrifuge briefly.
11. Incubate at 30°C for 3 h.

    **IMPORTANT**: Sample incubation for 2 h generates sufficient DNA for PCR-free library prep. Incubation of 1 h is also possible but leads to reduced yields and may not be appropriate for all types of cells. After incubation at 30°C, heat the heating block up to 65°C if the same heating block will be used in step 12.

    **NOTE**: If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.

12. Inactivate REPLI-g sc DNA Polymerase at 65°C for 3 min.
13. If not being used immediately afterwards, store amplified DNA either at 4°C for short-term storage, or at –20°C for long-term storage. DNA amplified using the REPLI-g Single Cell Kit

should be treated as genomic DNA and should undergo minimal freeze-thaw cycles. We recommend storage of nucleic acids at a concentration of at least 100 ng/µl.

14. Use the amount of REPLI-g amplified DNA diluted in water or TE buffer as stated in the manufacturer's instructions. If performing PCR analysis, dilute an aliquot of amplified DNA 1:50, and use 2 µl of diluted DNA for each PCR reaction.
15. Amplified DNA behaves like purified genomic DNA and has an approximate length of 2000 bp up to 70,000 bp. It is highly suited for use in a variety of downstream applications, including next-generation sequencing, array CGH and quantitative PCR.

    **NOTE**: Optical density (OD) measurements overestimate REPLI-g amplified DNA and should not be used. Typical DNA yields are approximately 30 µg per 50 µl reaction. Refer to Appendix A in the *REPLI-g Single Cell Handbook* for an accurate method of quantifying REPLI-g amplified DNA.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from **www.qiagen.com/literature**.

Safety data sheets (SDS) for any QIAGEN product can be downloaded from **www.qiagen.com/safety**.

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